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Abstract
Soybean cyst nematode infestation continues to be a serious agricultural problem. As part of an interdisciplinary effort to identify a biorational solution to the problem, analogs of glycinoeclepin A, a natural hatching stimulus of the nematode, were prepared and tested. Several of the analogs were discovered to inhibit the hatching of soybean cyst nematode eggs. On the basis of the results of egg hatch tests, the minimum functionality for egg hatch inhibition appears to be a keto diacid.

Keywords
Nematode, hatching inhibitor, synthesis

Comments
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Synthesis and Testing of Compounds That Inhibit Soybean Cyst Nematode Egg Hatch

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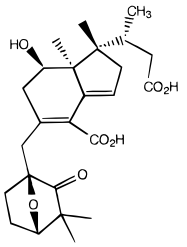
Soybean cyst nematode infestation continues to be a serious agricultural problem. As part of an interdisciplinary effort to identify a biorational solution to the problem, analogs of glycinoeclepin A, a natural hatching stimulus of the nematode, were prepared and tested. Several of the analogs were discovered to inhibit the hatching of soybean cyst nematode eggs. On the basis of the results of egg hatch tests, the minimum functionality for egg hatch inhibition appears to be a keto diacid.

Keywords: *Nematode; hatching inhibitor; synthesis*

The soybean cyst nematode (SCN), *Heterodera glycines*, has been a serious national plant disease problem for over 3 decades. It is spreading rapidly throughout the soybean-producing regions of the United States. In Iowa, the last 5 years have witnessed a dramatic spread of SCN. Although a number of strategies for the management of SCN have been advanced, a solution which is both economically feasible and biorational has not yet been found (Niblack et al., 1992). For example, nematicides and host resistance have been recommended for the management of plant-parasitic nematodes. However, the use of nematicides which are not readily biodegradable is not consistent with the principles of sustainable agriculture, and thus, many inexpensive and effective nematicides have been banned. The use of host resistance, however, is an environmentally safe management strategy but has problems as well. Prolonged use of SCN-resistant soybean varieties may lead to development of SCN races which can readily reproduce on resistant soybeans. Integration of 1 year of a SCN-resistant soybean variety with 2 years of nonhost crops into 3 year crop rotations is effective in managing SCN population densities, but market considerations make this strategy unattractive, if not unfeasible, to growers.

Since 1989, thousands of soil samples from Iowa and several adjacent north central states have been tested for SCN by the Cooperative Extension Service at Iowa State University, and ca. 75% were found to be infested. Furthermore, a great majority of the soil samples contained SCN densities >1000 eggs/100 cm^3 of soil. Significant yield suppression has been shown to occur at SCN population densities of 50−100 eggs/100 cm^3 of soil (Niblack et al., 1992). This nematode is rapidly becoming a major factor in limiting soybean yields throughout the United States.

Glycinoeclepin A is a hatching stimulus capable of initiating hatching of SCN eggs at concentrations as low as 10^{-12} g/mL (Masamune et al., 1982). It is a naturally occurring compound which should be readily biodegradable. Glycinoeclepin A has been isolated by extraction from kidney bean roots, but only milligram quantities were obtained from thousands of kilograms of roots (Masamune et al., 1982).



Glycinoeclepin A

Initially, our research began with the synthesis of analogs of glycinoeclepin A. These analogs were evaluated using an in vitro egg hatch assay (Wong et al., 1993). In the course of this work, we have discovered a number of compounds that very effectively inhibited the hatching of SCN eggs and a few that mildly accelerated the hatch. In this paper we will discuss the synthesis and testing of compounds that inhibit the hatch of SCN eggs.

The synthesis of selected diacids is shown below. The readily available keto ester **2** (Stork et al., 1963) was condensed with diethyl oxalate and potassium *tert*-butoxide to produce a diester which was reacted with glyoxylic acid according to the method of Bonadies and Scarpati and then hydrolyzed with lithium hydroxide in methanol to furnish diacid **3**. Diacid **5** was produced by condensation of diethyl oxalate with keto ester **2** followed by hydrolysis of the esters with lithium hydroxide in methanol.

Preliminary SCN egg hatch tests showed that **3** and **5** were equally effective inhibitors. In order to better understand the effect of changes in structure on inhibition of SCN egg hatch, we then prepared compounds **7** and **9** using procedures analogous to those used to prepare diacid **5**. Careful evaluation using an in vitro egg hatch assay (Wong et al., 1993) showed that acids **3**, **5**, **7,** and **9** all were reproducible inhibitors of SCN egg hatch. The corresponding diesters were not inhibitors. Keto esters **2** and **6** and keto acid **8** were not inhibitors. The minimum functionality for egg hatch inhibition appears to be a keto diacid. The hatch assay results are shown in Table 1. Interestingly, **7** and **9** are much less efficacious than **3** and **5**.

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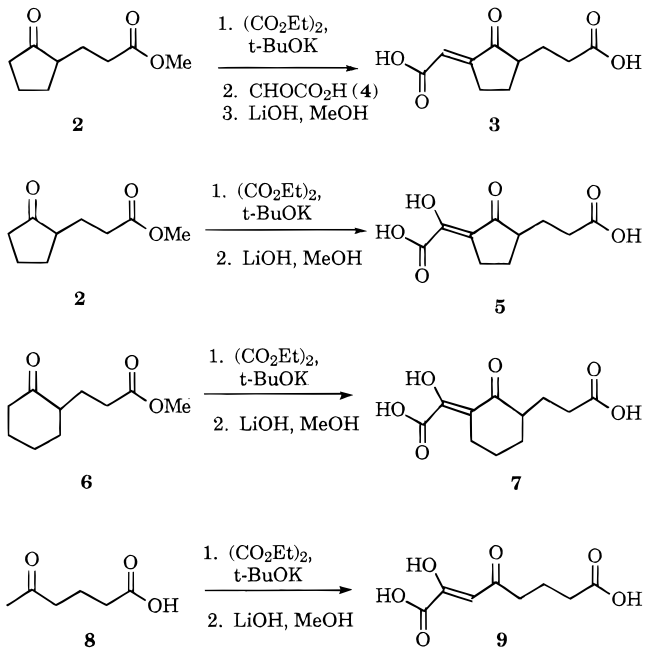

The keto diacids described herein are readily available and represent promising leads in the quest for safe and effective solutions to reducing SCN infestations. Chemicals that inhibit SCN egg hatch should be effective in managing the soybean cyst nematode in infested fields where soybeans are grown. The synthesis and testing of the heretofore-unknown keto diacids **3**, **5**, **7**, and **9** give testimony to the importance of a multidisciplinary approach to the solution of modern agricultural problems.

EXPERIMENTAL PROCEDURES

All starting materials used were of reagent purity and used as supplied. THF was dried over sodium and benzophenone. ^{1}H NMR data were obtained on a 300 MHz Nicollet spectrometer, and $CDCl_3$ was used as the solvent. All IR data were collected using NaCl plates. All purification was performed by flash chromatography using silica gel and *n*-hexane/ethyl acetate as eluent. The purity of all title compounds was determined to be >95% by 300 MHz proton NMR and/or elemental analysis.

Compound 3. The keto ester **2** (0.300 g, 1.76 mmol) was combined with 0.282 g (1.94 mmol, 1.1 equiv) of diethyl oxalate and cooled to 0 °C. In a separate flask, 0.299 g (2.46 mmol, 1.4 equiv) of potassium *tert*-butoxide was suspended in 3.5 mL of dry THF and cooled to 0 °C. The keto-ester/diethyl oxalate mixture was added dropwise over 1 h via syringe pump to the stirring THF solution at 0 °C. The reaction mixture was allowed to stir overnight and then acidified with 6 M HCl to pH 2. The solution was diluted with 3.5 mL of water and extracted three times with CH_2Cl_2. The combined organic layers were dried over Na_2SO_4 and concentrated in vacuo. Flash chromatography on silica gel, eluting with *n*-hexane/ethyl acetate (4:1), gave 0.430 g (86%) of the bis-ester.

To a solution of 0.231 g (2.5 mmol, 2.5 equiv) of glyoxalic acid monohydrate in 3.5 mL of 0.5 M Na_2HPO_4 and 1.5 mL of 1 M KH_2PO_4 cooled to 0 °C was added 0.300 g (1.05 mmol, 1 equiv) of the bis-ester dropwise along with an additional 2 mL of 0.5 M Na_2HPO_4 to maintain pH = 6.5. The reaction mixture was stirred overnight at 0 °C, the reaction quenched with 6 M HCl, and the mixture diluted with water and extracted three times with CH_2Cl_2. The combined organic layers were dried over Na_2SO_4 and concentrated on a rotary evaporator. The crude product was again dissolved in CH_2Cl_2 and made basic to pH 11 with 15% NaOH. The mixture was washed three times with diethyl ether, reacidified to pH 2 with 6 M HCl, extracted three times with CH_2Cl_2, dried over Na_2SO_4, and concentrated in vacuo yielding the pure compound (0.198 g, 75%).

Table 1. Hatching of Free Soybean Cyst Nematode Eggs in Experimental Compounds Relative to Hatching in Deionized Water at 25 °C in Vitro

		difference (%) from deionized water after	
compd	concn (μg/mL)	14 days	28 days
3	1	−4.4	−5.1
	54	−86.8	−87.3
5	5	+8.7	+4.1
	50	−81.2	−78.2
7	10	−13.4	−9.8
	100	−13.8	−19.4
9	10	−10.9	−11.8
	100	−19.3	−24.0

The keto ester (0.300 g, 1.25 mmol) was dissolved in 2.5 mL of methanol. To this solution werea added 0.240 g (10 mmol, 8 equiv) of lithium hydroxide and 0.280 g (5 mmol, 4 equiv) of potassium hydroxide. The mixture stirred for 48 h and was acidified with 6 M HCl to pH 2. The mixture was diluted with 2.5 mL of water and extracted three times with CH_2Cl_2. The combined organic layers were dried over Na_2SO_4 and concentrated in vacuo. The crude product was dissolved in CH_2Cl_2 and made basic to pH 11 with 15% NaOH. The mixture was washed three times with diethyl ether, reacidified to pH 2 with 6 M HCl, extracted three times with CH_2Cl_2, dried over Na_2SO_4, and concentrated on a rotary evaporator yielding the compound (0.167 g, 63%): 300 MHz ^{1}H NMR ($CDCl_3$) δ 6.65 (s, 1H), 2.52−2.47 (t, 1H), 2.38−2.00 (m, 4H), 1.84−1.50 (m, 4H); IR (neat) 3105 (m), 2972 (m), 2874 (m), 1725 (s), 1712 (s), 1462 (s), 1411 (m), 1290 (w), 1208 (m), 1167 (m), 943 (w), 919 (w) cm^{-1}.

Compound 5. The keto ester **2** (0.300 g, 1.76 mmol) was combined with 0.282 g (1.94 mmol, 1.1 equiv) of diethyl oxalate and cooled to 0 °C. In a separate flask, 0.299 g (2.46 mmol, 1.4 equiv) of potassium *tert*-butoxide was suspended in 3.5 mL of dry THF and cooled to 0 °C. The keto ester/diethyl oxalate mixture was added dropwise over 1 h via syringe pump to the stirring THF solution at 0 °C. The reaction mixture was allowed to stir overnight and then acidified with 6 M HCl to pH 2. The solution was diluted with 3.5 mL of water and extracted three times with CH_2Cl_2. The combined organic layers were dried over Na_2SO_4 and concentrated in vacuo. Flash chromatography on silica gel, eluting with *n*-hexane/ethyl acetate (4:1), gave 0.430 g (86%) of the bis-ester.

The bis-ester (0.300 g, 1.25 mmol) was dissolved in 2.5 mL of methanol. To this solution were added 0.240 g (10 mmol, 8 equiv) of lithium hydroxide and 0.280 g (5 mmol, 4 equiv) of potassium hydroxide. The mixture stirred for 48 h and was acidified with 6 M HCl to pH 2. The mixture was diluted with 2.5 mL of water and extracted three times with CH_2Cl_2. The combined organic layers were dried over Na_2SO_4 and concentrated in vacuo. The crude product was again dissolved in CH_2Cl_2 and made basic to pH 11 with 15% NaOH. The mixture was washed three times with diethyl ether, reacidified to pH 2 with 6 M HCl, extracted three times with CH_2Cl_2, dried over Na_2SO_4, and concentrated in vacuo yielding the compound (0.251 g, 95%): 300 MHz ^{1}H NMR ($CDCl_3$) δ 2.52−2.47 (t, 1H), 2.38−2.00 (m, 4H), 1.84−1.50 (m, 4H); IR (neat) 3100 (m), 2961 (m), 2876 (m), 1735 (s), 1714 (s), 1454 (s), 1408 (m), 1295 (w), 1201 (m), 1160 (m), 933 (w), 917 (w) cm^{-1}. Anal. Calcd for $C_{10}H_{12}O_6$: C, 52.63; H, 5.30; O, 42.07. Found: C, 52.45; H, 5.23; O, 42.32.

Compound 7. The keto ester **6** (0.300 g, 1.63 mmol) was combined with 0.262 g (1.79 mmol, 1.1 equiv) of diethyl oxalate and cooled to 0 °C. In a separate flask, 0.274 g (2.45 mmol, 1.5 equiv) of potassium *tert*-butoxide was suspended in 3.5 mL of dry THF and cooled to 0 °C. The keto ester/diethyl oxalate mixture was added dropwise over 1 h via syringe pump to the stirring THF solution at 0 °C. The reaction mixture was allowed to stir overnight and then acidified with 6 M HCl to pH 2. The solution was diluted with 3.5 mL of water and

extracted three times with CH_2Cl_2. The combined organic layers were dried over Na_2SO_4 and concentrated in vacuo. Flash chromatography on silica gel, eluting with *n*-hexane/ethyl acetate (4:1), gave 0.394g (90%) of the bis-ester.

The bis-ester (0.300 g, 1.12 mmol) was dissolved in 2.5 mL of methanol. To this solution were added 0.214 g (8.95 mmol, 8 equiv) of lithium hydroxide and 0.251 g (4.48 mmol, 4 equiv) of potassium hydroxide. The mixture stirred for 48 h and was acidified with 6 M HCl to pH 2. The mixture was diluted with 2.5 mL of water and extracted three times with CH_2Cl_2. The combined organic layers were dried over Na_2SO_4 and concentrated in vacuo. The crude product was again dissolved in CH_2Cl_2 and made basic to pH 11 with 15% NaOH. The mixture was washed three times with diethyl ether, reacidified to pH 2 with 6 M HCl, extracted three times with CH_2Cl_2, dried over Na_2SO_4, and concentrated on a rotary evaporator yielding the compound (0.246 g, 97%) as a white solid: 300 MHz ^{1}H NMR ($CDCl_3$) δ 2.50−2.41 (m); IR (neat) 3095 (s), 2936 (s), 2863 (s), 1732 (s), 1699 (s), 1450 (s), 1419 (s), 1313 (s), 1227 (s), 1132 (m), 940 (w), 833 (w) cm^{-1}. Anal. Calcd for $C_{11}H_{14}O_6$: C, 54.54; H, 5.83; O, 39.63. Found: C, 54.25; H, 5.23; O, 40.52.

Compound 9. Commercially available (Aldrich Chemical) 4-acetylbutyric acid (**8**) (0.300 g, 2.31 mmol) was combined with 0.371 g (2.54 mmol, 1.1 equiv) of diethyl oxalate and cooled to 0 °C. In a separate flask, 0.620 g (5.07 mmol, 2.2 equiv) of potassium *tert*-butoxide was suspended in 4.6 mL of dry THF and cooled to 0 °C. The 4-acetylbutyric acid/diethyl oxalate mixture was added dropwise over 1 h via syringe pump to the stirring THF solution at 0 °C. The reaction mixture was allowed to stir overnight and then acidified with 6 M HCl to pH 2. The solution was diluted with 3.5 mL of water and extracted three times with CH_2Cl_2. The combined organic layers were dried over Na_2SO_4 and concentrated in vacuo. Flash chromatography on silica gel, eluting with *n*-hexane/ethyl acetate (3:1), gave 0.450g (91%) of the keto acid.

The keto acid (0.300 g, 1.4 mmol) was dissolved in 2.5 mL of methanol. To this solution were added 0.134 g (5.6 mmol, 4 equiv) of lithium hydroxide and 0.157 g (2.8 mmol, 2 equiv) of potassium hydroxide. The mixture stirred for 48 h and was acidified with 6 M HCl to pH 2. The mixture was diluted with 2.5 mL of water and extracted three times with CH_2Cl_2. The combined organic layers were dried over Na_2SO_4 and concentrated in vacuo. The crude product was dissolved in CH_2Cl_2 and made basic to pH 11 with 15% NaOH. The mixture was washed three times with diethyl ether, reacidified to pH 2 with 6 M HCl, extracted three times with CH_2Cl_2, dried over Na_2SO_4, and concentrated in vacuo yielding the compound (0.269 g, 95%): 300 MHz ^{1}H NMR ($CDCl_3$) ∂ 2.56−2.51 (t, 2H), 2.42−2.37 (t, 2H), 2.15 (s, 2H), 1.95−1.85 (m, 2H); IR (neat) 3161 (s), 2940 (s), 2670 (s), 1734 (s), 1686 (s), 1653 (m), 1453 (s), 1418 (s), 1363 (s), 1285 (s), 1160 (s), 1069 (m), 947 (m), 840 (m), 742 (m), 667 (w) cm^{-1}. Anal. Calcd for $C_8H_{10}O_6$: C, 44.69; H, 4.29; O, 51.02. Found: C, 44.56; H, 4.45; O, 50.99.

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